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L10 ANSWER 1 OF 5 LIFESCI COPYRIGHT 2005 CSA on STN
ACCESSION NUMBER: 2004:90011 LIFESCI
TITLE: **Huntingtin** Bodies Sequester Vesicle-Associated Proteins by a Polyproline-Dependent Interaction
AUTHOR: Qin, Z.-H.; Wang, Y.; Sapp, E.; Cuiffo, B.; Wanker, E.; Hayden, M.R.; Kegel, K.B.; Aronin, N.; DiFiglia, M.
CORPORATE SOURCE: Laboratory of Cellular Neurobiology, Massachusetts General Hospital and Harvard Medical School, Charlestown, Massachusetts 02129, USA
SOURCE: Journal of Neuroscience [J. Neurosci.], (20040107) vol. 24, no. 1, pp. 269-281.
ISSN: 0270-6474.
DOCUMENT TYPE: Journal
FILE SEGMENT: N3
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Polyglutamine expansion in the N terminus of **huntingtin** (htt) causes selective neuronal dysfunction and cell death by unknown mechanisms. Truncated htt expressed in vitro produced htt immunoreactive cytoplasmic bodies (htt bodies). The fibrillar core of the mutant htt body resisted protease treatment and contained cathepsin D, ubiquitin, and heat shock protein (HSP) 40. The shell of the htt body was composed of globules 14-34 nm in **diameter** and was protease sensitive. HSP70, proteasome, dynamin, and the htt binding partners htt interacting protein 1 (HIP1), SH3-containing Grb2-like protein (SH3GL3), and 14.7K-interacting protein were reduced in their normal location and redistributed to the shell. Removal of a series of prolines adjacent to the polyglutamine region in htt blocked formation of the shell of the htt body and redistribution of dynamin, HIP1, SH3GL3, and proteasome to it. Internalization of transferrin was impaired in cells that formed htt bodies. In cortical neurons of Huntington's disease patients with early stage pathology, dynamin immunoreactivity accumulated in cytoplasmic bodies. Results suggest that accumulation of a nonfibrillar form of mutant htt in the cytoplasm contributes to neuronal dysfunction by sequestering proteins involved in vesicle trafficking.

L10 ANSWER 2 OF 5 BIOTECHNO COPYRIGHT 2005 Elsevier Science B.V. on STN
DUPLICATE
ACCESSION NUMBER: 2003:36389706 BIOTECHNO
TITLE: Aggregate formation and the impairment of long-term synaptic facilitation by ectopic expression of mutant **huntingtin** in Aplysia neurons
AUTHOR: Lee J.-A.; Lim C.-S.; Lee S.-H.; Kim H.; Nukina N.; Kaang B.-K.
CORPORATE SOURCE: B.-K. Kaang, Inst. of Molec. Biology and Genetics, Seoul National University, San 56-1 Silim-dong, Kwanak-gu, Seoul 151-742, South Korea.
E-mail: kaang@snu.ac.kr
SOURCE: Journal of Neurochemistry, (2003), 85/1 (160-169), 55 reference(s)
CODEN: JONRA0 ISSN: 0022-3042
DOCUMENT TYPE: Journal; Article
COUNTRY: United Kingdom
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 2003:36389706 BIOTECHNO
AB Huntington's disease (HD) is caused by an expansion of a polyglutamine (polyQ) tract within **huntingtin** (htt) protein. To examine the cytotoxic effects of polyQ-expanded htt, we overexpressed an enhanced green fluorescent protein (EGFP)-tagged N-terminal fragment of htt with 150 glutamine residues (Nhtt150Q-EGFP) in Aplysia neurons. A combined confocal and electron microscopic study showed that Aplysia neurons expressing Nhtt150Q-EGFP displayed numerous abnormal aggregates (

diameter 0.5-5 μm) of filamentous structures, which were formed rapidly (approximately 2 h) but which were sustained for at least 18 days in the cytoplasm. Furthermore, the overexpression of Nhtt150Q-EGFP in sensory cells impaired 5-hydroxytryptamine (5-HT)-induced long-term synaptic facilitation in sensori-motor synapses without affecting basal synaptic strength or short-term facilitation. This study demonstrates the stability of polyQ-based aggregates and their specific effects on long-term synaptic plasticity.

L10 ANSWER 3 OF 5 BIOTECHNO COPYRIGHT 2005 Elsevier Science B.V. on STN
DUPLICATE
ACCESSION NUMBER: 2002:34411593 BIOTECHNO
TITLE: Amyloid fibers are water-filled nanotubes
AUTHOR: Perutz M.F.; Finch J.T.; Berriman J.; Lesk A.
CORPORATE SOURCE: M.F. Perutz, Med. Res. Cncl. Lab. of Molec. Biol., Cambridge CB2 2QH, United Kingdom.
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (16 APR 2002), 99/8 (5591-5595), 17 reference(s)
CODEN: PNASA6 ISSN: 0027-8424
DOCUMENT TYPE: Journal; Conference Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 2002:34411593 BIOTECHNO
AB A study of papers on amyloid fibers suggested to us that cylindrical β-sheets are the only structures consistent with some of the x-ray and electron microscope data. We then found that our own 7-year-old and hitherto enigmatic x-ray diagram of poly-L-glutamine fits a cylindrical sheet of 31 Å **diameter** made of β-strands with 20 residues per helical turn. Successive turns are linked by hydrogen bonds between both the main chain and side chain amides, and side chains point alternately into and out of the cylinder. Fibers of the exon-1 peptide of **huntingtin** and of the glutamine- and asparagine-rich region of the yeast prion Sup35 give the same underlying x-ray diagrams, which show that they have the same structure. Electron micrographs show that the 100-Å-thick fibers of the Sup35 peptide are ropes made of three protofibrils a little over 30 Å thick. They have a measured mass of 1,450 Da/Å, compared with 1,426 Da/Å for a calculated mass of three protofibrils each with 20 residues per helical turn wound around each other with a helical pitch of 510 Å. Published x-ray diagrams and electron micrographs show that fibers of synuclein, the protein that forms the aggregates of Parkinson disease, consist of single cylindrical β-sheets. Fibers of Alzheimer Aβ fragments and variants are probably made of either two or three concentric cylindrical β-sheets. Our structure of poly-L-glutamine fibers may explain why, in all but one of the neurodegenerative diseases resulting from extension of glutamine repeats, disease occurs when the number of repeats exceeds 37-40. A single helical turn with 20 residues would be unstable, because there is nothing to hold it in place, but two turns with 40 residues are stabilized by the hydrogen bonds between their amides and can act as nuclei for further helical growth. The Aβ peptide of Alzheimer's disease contains 42 residues, the best number for nucleating further growth. All these structures are very stable; the best hope for therapies lies in preventing their growth.

L10 ANSWER 4 OF 5 BIOTECHNO COPYRIGHT 2005 Elsevier Science B.V. on STN
DUPLICATE
ACCESSION NUMBER: 1998:28491576 BIOTECHNO
TITLE: Association of HAP1 isoforms with a unique cytoplasmic structure
AUTHOR: Li S.-H.; Gutekunst C.-A.; Hersch S.M.; Li X.-J.
CORPORATE SOURCE: Dr. X.-J. Li, Department of Genetics, Emory University

School of Medicine, 1462 Clifton Rd. N.E., Atlanta, GA 30322, United States.
SOURCE: Journal of Neurochemistry, (1998), 71/5 (2178-2185), 30 reference(s)
CODEN: JONRA0 ISSN: 0022-3042
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1998:28491576 BIOTECHNO
AB HAP1 is a neural protein and interacts with the Huntington's disease protein **huntingtin**. There are at least two HAP1 isoforms, HAP1-A and HAP1-B, which have different C-terminal amino acid sequences. Here we report that both HAP1 isoforms associate with a unique cytoplasmic structure in neurons of rat brain. The HAP1-immunoreactive structure appears as an inclusion that is an oval mass of electron-dense material, 0.5-3 μm in **diameter**, containing many curvilinear or ring-shaped segments, and often containing electron- lucent cores. This structure is very similar to those previously termed the stigmoid body, nematosome, or botrysome. Transfection of cell lines with cDNA encoding HAP1-A, but not HAP1-B, resulted in similar HAP1-immunoreactive inclusions in the cytoplasm, suggesting that HAP1-A is essential to the formation of this structure. Yeast two-hybrid and transfection studies show that both HAP1-A and HAP1-B can self-associate, implying that native HAP1 in the cytoplasmic inclusion may be a heteromultimer of HAP1-A and HAP1-B. Coexpression of HAP1-A and HAP1-B in human embryonic kidney 293 cells demonstrates that the ratio of the expressed HAP1-A to HAP1-B regulates the formation of HAP1-associated inclusions. We propose that HAP1 isoforms are involved in the formation of HAP1-immunoreactive inclusions in the neuronal cytoplasm.

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ACCESSION NUMBER: 1998-0409800 PASCAL
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TITLE (IN ENGLISH): Wild-type and mutant **huntingtins** function in vesicle trafficking in the secretory and endocytic pathways
AUTHOR: VELIER J.; MANHO KIM; SCHWARZ C.; TAE WAN KIM; SAPP E.; CHASE K.; ARONIN N.; DIFIGLIA M.
CORPORATE SOURCE: Department of Neurology, Massachusetts General Hospital, Boston, Massachusetts 02114, United States; Medicine and Cell Biology, University of Massachusetts Medical Center, Worcester, Massachusetts 01655, United States
SOURCE: Experimental neurology, (1998), 152(1), 34-40, 39 refs.
ISSN: 0014-4886 CODEN: EXNEAC
DOCUMENT TYPE: Journal
BIBLIOGRAPHIC LEVEL: Analytic
COUNTRY: United States
LANGUAGE: English
AVAILABILITY: INIST-9181, 354000072787170040
AN 1998-0409800 PASCAL
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AB **Huntingtin** is a cytoplasmic protein that is found in neurons and somatic cells. In patients with Huntington's disease (HD), the NH.sub.2-terminal region of **huntingtin** has an expanded polyglutamine tract. An abnormal protein interaction by mutant **huntingtin** has been proposed as a mechanism for HD pathogenesis. **Huntingtin** associates with vesicle membranes and interacts with proteins involved in vesicle trafficking. It is unclear where along

vesicle transport pathways wild-type and mutant **huntingtins** are found and whether polyglutamine expansion affects this localization. To distinguish wild-type and mutant **huntingtin**, fibroblasts from normals and HD patients with two mutant alleles (homozygotes) were examined. Immunofluorescence confocal microscopy showed that mutant **huntingtin** localized with clathrin in membranes of the trans Golgi network and in clathrin-coated and noncoated endosomal vesicles in the cytoplasm and along plasma membranes. Separation of organelles in Nycodenz gradients showed that in normal and HD homozygote patient cells, **huntingtin** was present in membrane fractions enriched in clathrin. Similar results were obtained in fibroblasts from heterozyote juvenile HD patients who had a highly expanded polyglutamine tract in the HD allele. Western blot analysis of membrane fractions from rat brain showed that wild-type **huntingtin** was present in fractions that contained purified clathrin-coated membranes or a mixture of clathrin-coated and noncoated membranes. Electron microscopy of **huntingtin** immunoreactivity in rat brain revealed labeling along dendritic plasma membranes in association with clathrin-coated pits and clusters of noncoated endosomal vesicles 40-60 nm in **diameter**. These data suggest that wild-type and mutant **huntingtin** can influence vesicle transport in the secretory and endocytic pathways through associations with clathrin-coated vesicles.

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 273, No. 44, Issue of October 30, pp. 28873–28877, 1998
Printed in U.S.A.

Expression of Polyglutamine-expanded Huntingtin Activates the SEK1-JNK Pathway and Induces Apoptosis in a Hippocampal Neuronal Cell Line*

(Received for publication, June 17, 1998, and in revised form, September 8, 1998)

Ya Fang Liu‡

From the Department of Pharmaceutical Sciences, Northeastern University, Boston, Massachusetts 02115

Huntington's disease is one of a growing number of hereditary neurodegenerative disorders caused by expansion of a polyglutamine stretch at the NH_2 terminus of huntingtin. To explore whether polyglutamine-expanded huntingtin induces neuronal toxicity, I examined the expression of the full-length of huntingtin with 16, 48, or 89 polyglutamine repeats in a rat hippocampal neuronal cell (HN33). Expression of mutated huntingtin with 48 or 89 polyglutamine repeats stimulated c-Jun amino-terminal kinases (JNKs) activity and induced apoptotic cell death in HN33 cells while expression of normal huntingtin with 16 polyglutamine repeats had no toxic effect. The JNK activation precedes apoptotic cell death and co-expression of a dominant negative mutant form of stress-signaling kinase (SEK1) nearly completely blocked activation of JNKs and neuronal apoptosis mediated by mutated huntingtin. Taken together, my studies demonstrate that expression of polyglutamine-expanded huntingtin induces neuronal apoptosis via activation of the SEK1-JNK pathway.

Huntington's disease (HD)[1] is a progressive neurodegenerative disorder with an autosomal dominant inheritance (1). The genetic defect of the HD gene involves an expansion of a CAG repeat where normal and expanded HD allele sizes range between 6 to 37 and 34 to 121, respectively (1–4). The most common pathologic allele in the HD gene has between 40 and 49 CAG repeats and is clinically manifested by middle age onset with choreiform movement, mental impairment, and cognitive dysfunction due to a selective loss of striatal, cortical, and hippocampal neurons (1–4). Juvenile HD, associated with a longer CAG repeat, is characterized by a severe and diffuse loss of neurons throughout the brains (1–4).

Although the HD gene has been identified for several years, the molecular mechanism by which mutated huntingtin induces neurons to die remains unclear. Many studies have suggested that glutamate-mediated excitotoxicity may play an essential role in the pathogenesis of HD (7). Administration of NMDA receptor agonists such as quinolinic acid induces a behavioral phenotype and neuropathology strikingly similar to that observed in HD patients (8). Apparently, polyglutamine repeat-expanded huntingtin and NMDA receptors may stimulate a common neurotoxic pathway to cause neuronal injury. Therefore, identification of such a neurotoxic pathway may be not only crucial for understanding of the mechanism of neuronal death induced by mutated huntingtin and glutamate, but also for identifying potential drug targets for the treatment of HD as well as other neurodegenerative diseases.

Activation of JNKs has also been implicated in neuronal death induced by a wide range of environmental stress stimuli and glutamate-mediated excitotoxicity. In both primary cultured sympathetic neurons and differentiated pheochromocytoma PC12 cells, deprivation of nerve growth factor leads to the JNK activation and neuronal apoptosis (9, 10). In primary cultured striatal neurons, stimulation of NMDA receptors leads to activation of JNKs and increases of c-Jun and c-Fos expression (11). Intriguingly, gene-targeted knockout of JNK3, an isoform of JNKs that is almost exclusively expressed in the brain, results in a remarkable resistance to neuronal excitotoxicity induced by kainic acid (12). Seizures and hippocampal neuronal apoptosis are prevented, while phosphorylation of c-Jun and the transcription activity of AP-1 are markedly reduced in the JNK3 (−/−) mice (12).

The present study was undertaken to determine whether expression of full-length polyglutamine expanded huntingtin induces neuronal toxicity and to explore the potential molecular mechanism of neuronal death. I found that expression of full-length huntingtin with 48 or 89 polyglutamine repeats resulted in the JNK activation and apoptotic cell death of an immortalized rat hippocampal neuronal cell line (HN33). Co-expression of a dominant negative form of SEK1 nearly completely blocked mutated huntingtin-mediated neuronal apoptosis. Thus, I conclude that polyglutamine-expanded huntingtin, via activation of JNKs mediates neuronal apoptosis.

EXPERIMENTAL PROCEDURES

Construction of Expression Vectors for Full-length Huntingtin with 16, 48, or 89 CAG Repeats—The first third of the full-length construct was derived by ligation of IT16LL (bp 932–3018) with three different PCR products (bp 2401–3270, bp 637–1429, and 187–858). A 3027-bp cDNA fragment was lifted from this resulting construct and ligated to corresponding sites in the cDNA clone IT15B (bp 3024–10366). The CAG repeat size in this full-length huntingtin construct, pFL16HD, is 16. PCR products were generated from the genomic DNA of an adult patient with 48 CAG repeats and a juvenile onset case with 89 CAG repeats. These PCR products were ligated to replace the corresponding region in pFL16HD to generate the pFL48HD and pFL89HD with 48 and 89 CAG repeats, respectively. Colony hybridization and PCR were used to identify the 48 and 89 CAG huntingtin clones, and positive clones were verified by DNA sequence analysis.

Cell Culture and Transient Expression of Normal and Polyglutamine-expanded Huntingtin—HN33 cells, cultured in 10-cm plates were initially maintained in DMEM medium supplemented with 10% of fetal bovine serum. After 20–30 passages, cells became differentiated, exhibiting neurite outgrowth. The medium was switched to DMEM/

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[1] The abbreviations used are: HD, Huntington's disease; JNK, c-Jun amino-terminal kinase; SEK1, stress-signaling kinase 1; NMDA, *N*-methyl-D-aspartate; PCR, polymerase chain reaction; GST, glutathione *S*-transferase; TUNEL, Tdt-mediated dUTP-biotin nick end labeling; ICE, interleukin 1β converting enzyme; bp, base pair(s); DMEM, Dulbecco's modified Eagle's medium.

F-12 (50/50) in which the morphology of HN33 cells remains unaltered after over 100 passages. 50–60% confluent HN33 or 293 cells were washed once with serum-free medium prior to transfection. Transfection was performed using Lipofectin (Boehringer Mannheim) according to the manufacturer's instructions, and fetal bovine serum was added to the medium 12 h after transfection to a final concentration of 1%. 60 μg of plasmid with 10 μl of Lipofectin/60-mm plate was used in all transfection experiments. After 24 h, the transfection medium was removed and replaced by fresh medium with 1% fetal bovine serum.

Western Blotting—48–72 h after transfection, 293 cells were harvested and lysed in 1% Nonidet P-40 lysis buffer (14). For immunoprecipitation experiments, cell lysates were incubated with affinity-purified anti-NH_2 terminus huntingtin polyclonal antibody 437 for 4–6 h (14). Cell lysates or precipitated proteins were resolved on SDS-polyacrylamide gel electrophoresis, transferred, and immunoblotted with anti-huntingtin monoclonal antibody 4C8 (15) as indicated in figure legends.

TUNEL Assay—2×10^4 HN33 cells were plated on a slide culture chamber. Transient transfection of different plasmids was conducted using Lipofectin. zVAD-fmk (16) or zDEVD-fmk (17) (Enzyme Systems Products, Livermore, CA) was added to the medium during transfection. Transfection medium was removed at the time indicated in the figures, and cells were washed once with serum-free medium, fixed with 4% paraformaldehyde, and then permeabilized with 0.1% of Triton X-100 for 2 min. The TUNEL staining (18) was performed as per the manufacturer's instructions (Boehringer Mannheim). Most apoptotic HN33 cells were detached from the slides. In Figs. 2*A* and 3*C*, TUNEL stain was performed on remaining attached cells. HN33 cells showing the retraction of neurites and positive stain in the nucleus were recognized as apoptotic. TUNEL stain negative cells (living cells) were counted in the × 20 power field in four different places on the slides, and about 600~800 control cells were counted.

JNK Assay—16 h after transfection, HN33 cells were lysed with 1% Triton buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EGTA. 1 mM EDTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, 1 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride). Cell lysates were incubated with GST-c-Jun (1–89) fusion proteins immobilized on glutathione-Sepharose beads to separate JNKs. These beads were resuspended in 30 μl of kinase buffer (25 mM Tris-HCl, 5 mM β-glycerophosphate, 2 mM dithiothreitol, 0.1 Na_3VO_4, and 10 mM $MgCl_2$). The kinase assay was performed at 30 °C for 30 min, and the reaction was stopped by adding SDS sample buffer. The samples were resolved in a SDS gel, transferred, and the blot was analyzed by Western blotting using a phospho (Ser-63)-specific c-Jun antibody (New England Biolabs).

RESULTS

To assess whether polyglutamine-expanded huntingtin causes neuronal toxicity, full-length huntingtin expression constructs containing 16, 48, or 89 CAG repeats, respectively, were generated by assembly of a combination of reverse transcription-PCR products from normal and human HD lymphoblast and plasmid cDNA clones IT16L and IT15B (1). To test whether the resulting constructs pFL16HD, pFL48HD, or pFL89HD express different huntingtins, they were transiently transfected into 293 embryonic kidney cells. Expression of either normal or polyglutamine-expanded huntingtin in 293 cells did not generate visible cell toxicity, and the rate of cell proliferation remained unaltered. Transfected cells were lysed 72 h after transfection, and the levels of huntingtin expression were analyzed by immunoblotting using an anti-huntingtin monoclonal antibody, 4C8 (15). As shown in Fig. 1, all three huntingtin constructs constitutively express the huntingtin protein. Similar to previous reports (15), mutant proteins migrate slightly slower than normal huntingtin (Fig. 1). Because transfection efficiency is about 40–50% according to β-galactosidase staining (data not shown), the amount of polyglutamine-expanded huntingtin expressed in these transfectants under an optional condition is about three to five times higher than that of endogenous huntingtin in 293 cells (Fig. 1).

Next, I examined whether expression of polyglutamine-expanded huntingtin induces neuronal toxicity. Since hippocampus is one of the brain regions affected in HD (2, 4), I used an

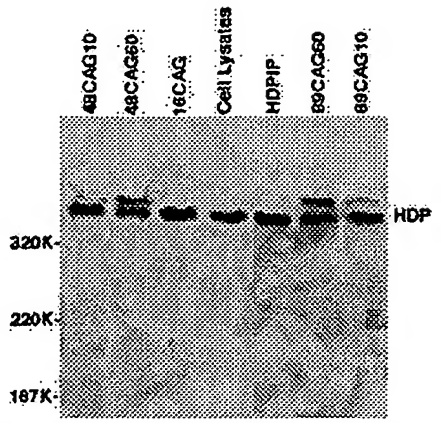


FIG. 1. **Detection of huntingtin expression by Western blotting.** 293 cells transfected with pFL16HD, pFL48HD, or pFL89HD were harvested and lysed 72 h after transfection, and the levels of huntingtin expression were analyzed by immunoblotting using an anti-huntingtin antibody 4C8. 48CAG10 and 48CAG60 are from 293 cell lysates transfected with 10 or 60 μg of pFL48HD/60-mm plate; 16CAG is from 293 cells transfected with 10 μg of pFL16HD/60-mm plate; HDPIP, immunoprecipitation of huntingtin with 437 from 293 cell lysates; 89CAG10 and 89CAG60 are from 293 cell lysates transfected with 10 or 60 μg pFL89HD/60-mm plate. Both 4C8 and 437 have been characterized and reported previously (14, 15).

immortalized rat hippocampal neuronal cell line, HN33, in my experiments (13). To normalize the expression level in different transfection experiments, 60 μg of plasmid DNA/60-mm plate and the same amount of Lipofectin were used in all experiments, and under this condition, the levels of expression of huntingtin were similar according to huntingtin immunoblotting. Transfection of pcDNA1 (vector) or normal huntingtin with 16 CAG repeats (pFL16HD) did not produce any toxic effect in HN33 cells, and DNA fragmentation was not detectable using TUNEL stain (Fig. 2*A*, *left* and *middle panels*), although expression of normal huntingtin slightly suppressed proliferation of HN33 cells (Fig. 2*A*, *middle panel*). Expression of mutated huntingtin with 48 or 89 polyglutamine repeats (pFL48HD or pFL89HD), however, clearly induced cell toxicity in HN33 cells. Apoptosis was initially observed between 20 and 24 h after transfection of pFL48HD or pFL89HD. At 48 h after transfection, about ~75% of HN33 cells were apoptotic. As shown in the *right panel* of Fig. 2*A*, 24 h after transfection of pFL48HD, a small population of HN33 cells was stained positively by TUNEL, and at 48 h after transfection of pFL48HD, most cells were detached from the slide, and most remaining attached cells exhibited DNA fragmentation. Although transfection efficiency is about 30–40%, according to β-galactosidase staining, about 90~95% of HN33 cells were apoptotic at 72 h after transfection of either pFL48HD or pFL89HD. A similar phenomenon has been observed following injection of the Rac target protein POSH into COS-1 cells, which lead to apoptosis of injected and noninjected neighboring cells (19). Since POSH is also a JNK activator, one possible explanations is that the JNK-activated apoptotic cells may produce and secrete a toxin that contributes to the death of neighboring cells. In addition, scattered neuronal cells in culture may be more vulnerable to neurotoxic stimuli.

Mutated huntingtin with 48 or 89 polyglutamine repeats correlates with middle age and juvenile onset of HD, respectively (5, 6). To examine whether mutated huntingtin with 89 polyglutamine repeats generates earlier and more severe neuronal toxicity than that induced by mutated huntingtin with 48 polyglutamine repeats, I performed a detailed time course of

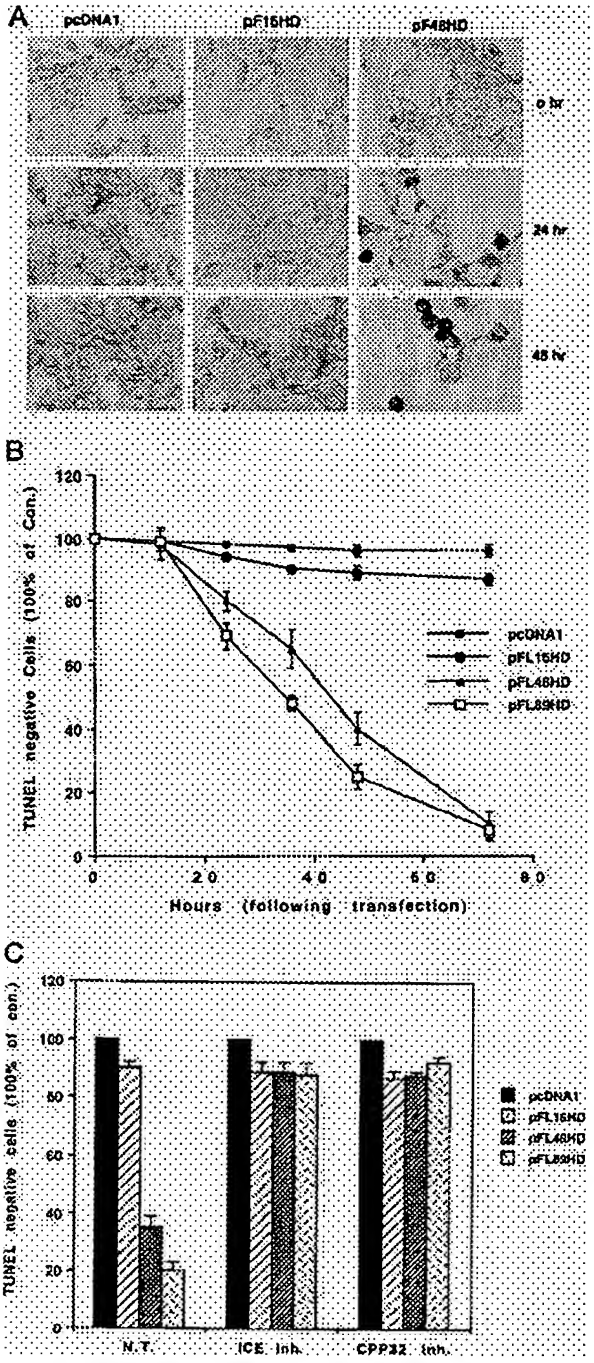


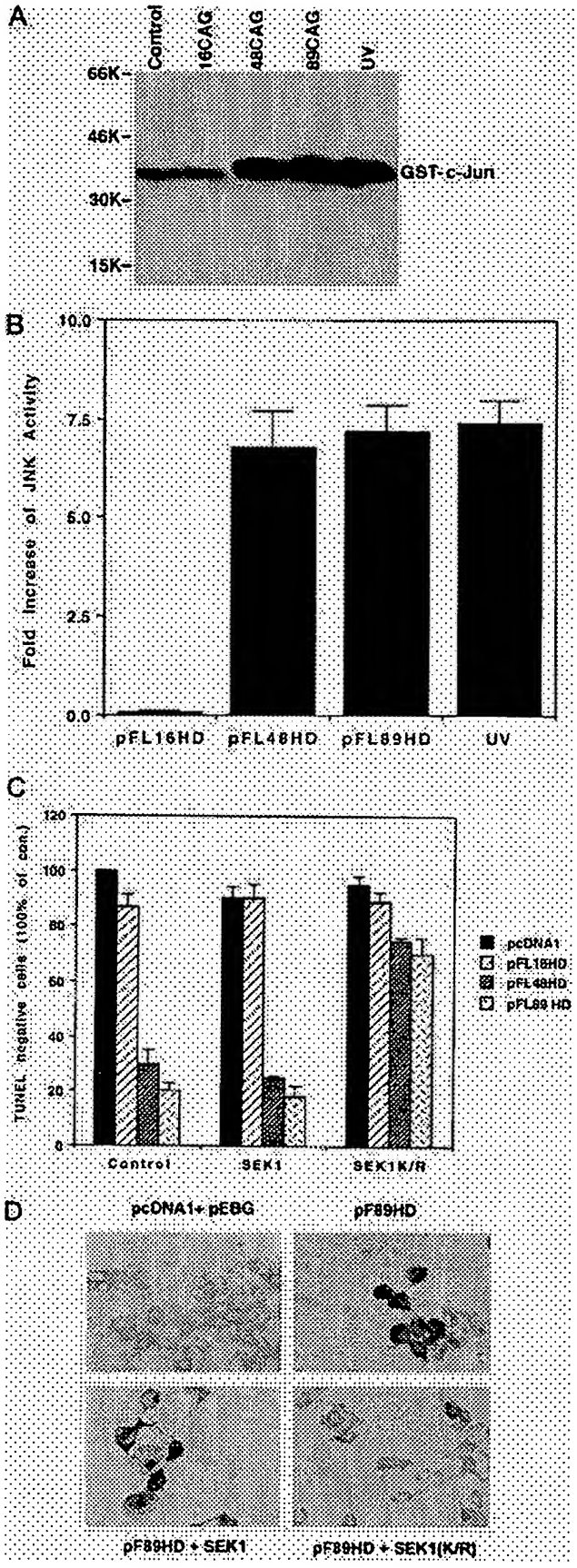
FIG. 2. **Expression of polyglutamine-expanded huntingtin induces apoptotic cell death in HN33 cells.** *A*, expression of mutated huntingtin with 48 CAG repeats mediates apoptosis. HN33 cells were transfected with pcDNA1, pFL16HD, or pFL48HD using Lipofectin. After transfection, cells were fixed at the time indicated in the figure followed by TUNEL staining. Data are from a typical experiment and have been reproduced twice with similar results. *B*, time course of expression of pcDNA1, pFL16HD, pFL48HD, or pHL89HD-induced apoptotic cell death of HN33 cells. HN33 cells were fixed and stained with TUNEL at the time indicated in the figure after transfection. TUNEL stain-negative cells were counted in the × 20 power field. The total number of 600~800 of TUNEL-negative cells in pcDNA1 transfectants is designated as 100%. Data are the average of four independent experiments. *C*, ICE or CPP32 inhibitors block cell death induced by mutated huntingtin. 10 μg/ml the ICE inhibitor zVAD-frm or CPP32 inhibitor zDEVD-frm was added to the medium during transfection. After 48 h, cells were fixed, TUNEL staining was performed, and TUNEL-negative cells were counted. Data are the average of three independent experiments.

HN33 cell survival following transfection with different huntingtin constructs. HN33 cells were transfected with pcDNA1, pFL16HD, pFL48HD, or pFL89HD, and after different times of transfection as indicated in Fig. 2*B*, cells were fixed and TUNEL-stained. Since most apoptotic cells were detached from the plates, TUNEL-negative cells were counted. Compared with transfection of pcDNA1 (control), transfection with normal huntingtin containing 16 polyglutamine repeats did not significantly reduce the number of TUNEL-negative cells between 12 and 72 h after transfection (Fig. 2*B*). As expected, neuronal toxicity induced by transfection with mutated huntingtin containing 89 polyglutamine repeats occurred 2–3 h earlier than that mediated by mutated huntingtin with 48 polyglutamine repeats (Fig. 2*B*).

To determine further whether polyglutamine-expanded huntingtin-induced cell death is apoptotic, an ICE inhibitor (zVAD-frm) or CPP32 inhibitor (zDEVD-frm) was added to the medium during transfection (16, 17). Both the ICE and CPP32 inhibitors completely blocked apoptotic cell death induced by expression of mutated huntingtin with 48 or 89 polyglutamine repeats. TUNEL staining was negative (Fig. 2*C*), and the rate of cell proliferation was similar to the control (transfected with pFL16HD) at both 48 and 72 h after transfection. ICE cleaves inactive CPP32 precursor, thereby activating the enzyme (20). This result therefore suggests that expression of polyglutamine-expanded huntingtin may stimulate ICE, which in turn activates CPP32 to induce apoptotic cell death.

I then explored the possible molecular mechanism of polyglutamine-expanded huntingtin-mediated neuronal toxicity. Since activation of JNK has been implicated in neuronal apoptosis induced by glutamate, kainic acid, or deprivation of neurotrophic factors (9–12), and since glutamate-mediated excitotoxicity may be involved in HD neuronal loss (7, 8), I investigated whether expression of polyglutamine-expanded huntingtin induces activation of JNKs. GST c-Jun (1–89 amino acids) was utilized as a substrate to measure JNK activity. HN33 cells were transfected with pcDNA1, pFL16HD, pFL48HD, or pFL89HD, and 16 h after transfection, cells were lysed, and JNK was precipitated by GST-c-Jun fusion protein beads. A low level of JNK activation was observed in pcDNA1-transfected cells (Fig. 3*A*, *Control*). This result is consistent with the data obtained from primary cultures of rat striatal neurons where a basal level of JNK activation was also observed (12). Transfection of normal huntingtin with 16 polyglutamine repeats did not further increase the amount of serine phosphorylated GST-c-Jun and thus did not stimulate JNK activity in HN33 cells (Fig. 3, *A* and *B*). Expression of mutated huntingtin with 48 or 89 polyglutamine repeats, however, significantly increased the level of JNK activity. As shown in Fig. 3, *A* and *B*, serine phosphorylated GST-c-Jun was increased 7–8-fold 16 h upon transfection of either pFL48HD or pFL89HD, similar to the level of JNK activity induced by 30 min of UV irradiation (Fig. 3, *A* and *B*). These results indicate that the polyglutamine repeat expansion of huntingtin enables it to activate JNKs in HN33 cells and the JNK activation precedes apoptotic cell death.

Next, I explored whether activation of JNK is responsible for polyglutamine-expanded huntingtin-induced apoptotic cell death in HN33 cells. JNK is specifically activated by SEK1 (21), and a dominant negative mutant form of SEK1 (K54R) can act as an inhibitor of the wild-type, blocking its phosphorylation and the activation of JNK (22, 23). To investigate the role of JNK activation in polyglutamine-expanded huntingtin-mediated apoptosis in HN33 cells, I examined the effect of co-expression of this dominant negative mutant form of SEK1 with polyglutamine-expanded huntingtin. Transient expres-



sion of either wild-type or dominant negative SEK1 alone had little effect on the proliferation and survival of HN33 cells (data not shown). Co-expression of pcDNA1 with pEBG (SEK1 vector) also did not produce any toxic effect (Fig. 3, *C* and 3*D*, *upper left panel*). Co-transfection of wild-type SEK1 with pFL48HD or pFL89HD did not affect neuronal toxicity induced by mutated huntingtin (Fig. 3*C*), while co-expression of dominant negative mutant SEK1, however, significantly prevented apoptotic cell death induced by the mutated huntingtin with either 48 or 89 polyglutamine repeats (Fig. 3, *C* and *D*). At 48 h after transfection, about 25–30% of cells had undergone apoptotic cell death compared with ~75% of cells after expression of mutated huntingtin with 48 or 89 polyglutamine repeats alone (Fig. 3, *C* and *D*). These TUNEL-negative HN33 cells appeared to be viable, because cells continued to proliferate, and trypan blue staining was negative. I further examined whether the rescuing effect of dominant negative SEK1 is mediated by inhibition of the JNK activation. Co-expression of dominant negative SEK1 significantly attenuated the JNK activity induced by mutated huntingtin with either 48 or 89 polyglutamine repeats (data not shown). These results further support the conclusion that polyglutamine-expanded huntingtin activates the SEK1-JNK pathway to mediate apoptosis in HN33 cells.

DISCUSSION

In the present study, I demonstrate that expression of mutated huntingtin containing 48 or 89 polyglutamine repeats activates the SEK1-JNK pathway and induces apoptosis in a hippocampal neuronal cell line. The observed neuronal toxicity is unlikely to be due to overexpression of mutated proteins, since expression of the same level of normal huntingtin with 16 polyglutamine repeats does not initiate cell death. Apparently, neuronal apoptosis induced by huntingtin is dependent on expansion of its polyglutamine repeat. Moreover, neuronal toxicity induced by mutated huntingtin with 89 polyglutamine repeats occurs slightly, but consistently, earlier than huntingtin with 48 polyglutamine repeats, further supporting the notion that neuronal apoptosis is mediated by polyglutamine repeat expansion. Since expression of mutated huntingtin alone does not induce apoptosis in 293 cells, this toxic effect may be a cell-specific event.

Selective loss of neurons in different brain regions is a hallmark of HD (2, 4), and neuronal apoptosis is one of the pathological changes observed in brains of HD patients, particularly in the early stage of the disease (24, 25). Thus, understanding of the molecular mechanism of neuronal death in HD is essential for revealing the pathogenesis of HD. My studies first demonstrate that expression of polyglutamine-expanded huntingtin leads to neuronal apoptosis. Since hippocampal neurons are targeted in HD (2, 4), my results are consistent with the major pathological feature of the disease. Moreover, the time

FIG. 3. **Mutated huntingtin-induced apoptosis is mediated by activation of the SEK1-JNK pathway.** *A*, JNK activation by mutated huntingtin. HN33 cells were transiently transfected with different plasmids as indicated in the figure. 16 h after transfection, HN33 cells were lysed, and JNK activity was measured as described under "Experimental Procedure." Data are from a typical experiment that has been repeated three times with similar results. *B*, increase of JNK activity was determined by analyzing the blots with a densitometer. The values depicted represent the -fold stimulation of JNK activity from different huntingtin-transfected cells over pcDNA1-transfected cells. Data are the average of three independent experiments. *C* and *D*, dominant negative SEK1 blocks neuronal toxicity induced by mutated huntingtin. *C*, after TUNEL staining, TUNEL-negative cells were counted, and the number of TUNEL-negative cells transfected with pcDNA1 was designated as 100%. Data are the average of three independent experiments. *D*, HN33 cells were co-transfected with different plasmids as indicated in the figure. Cells were fixed at 48 h after transfection followed by TUNEL staining.

course of neuronal toxicity generated by mutated huntingtin is clearly correlated with the length of the polyglutamine repeat as is the onset of the disease (5, 6).

Activation of JNK appears to be a major factor in the apoptotic death of HN33 cells induced by polyglutamine-expanded huntingtin. Mutated huntingtin with 48 or 89 polyglutamine repeats activates JNK in HN33 cells, while normal huntingtin with 16 repeats fails to do so, suggesting that activation of JNK by huntingtin requires expansion of the polyglutamine repeat. Activation of JNK induced by mutated huntingtin takes place several hours prior to apoptotic cell death, implying that JNK activation may trigger apoptotic pathways. The JNK-c-Jun-mediated apoptosis has been reported to be sensitive to both ICE and CPP32 inhibitors (19, 26), mutated huntingtin-mediated apoptosis was blocked by either inhibitor. Furthermore, dominant negative SEK1 inhibits mutated huntingtin induced-activation of JNKs in HN33 cells and also attenuates apoptotic cell death, further indicating that mutated huntingtin activates the SEK1-JNK pathway to induce neuronal apoptosis in HN33 cells.

Excitotoxicity is thought to contribute a final common pathway of neuronal injury in a wide range of neurodegenerative disorders including HD (7, 27). Administration of NMDA receptor agonists in rats causes selective loss of medium-spiny neurons in striatum that are also particularly affected in HD (8). The most intriguing data comes from gene-targeted knockout of JNK3, which renders mice resistant to neuronal excitotoxicity mediated by kainic acid receptors (12). Given the fact that activation of kainic acid or glutamate receptors and mutated huntingtin share selective neuronal targets, they could also utilize common cellular mediators for the induction of neuronal toxicity. JNK is one such cellular mediator for neuronal toxicity induced by both mutated huntingtin and glutamate/kainic acid receptors, since my current study and other reports (11, 12) show that activation of JNK is responsible for neuronal toxicity induced either by mutated huntingtin or by stimulation of glutamate/kainic acid receptors.

Currently, it is unclear whether JNK activation precedes neuronal loss in HD patients. JNK activates c-Jun and AP-1 transcription factors to induce apoptosis (9, 27). Increased expression and translocation of c-Jun and NF-κB (often concomitantly activated with the JNK cascade) has been observed in several neurodegenerative diseases (28, 29). Moreover, JNK activation is responsible for neuronal apoptosis induced by a variety of oxidative stress stimuli such as ischemia (30). These data and my current study suggest that overactivation of the JNK cascade may be a common pathway of neuronal death in different neurodegenerative diseases and in acute insults. In summary, my findings show that activation of the SEK1-JNK pathway may mediate neuronal death in HD, and proteins that are involved in activation of this signaling pathway may therefore be potential drug targets for the prevention of neuronal loss in HD as well as other neurodegenerative disorders.

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REFERENCES

1. The Huntington's Disease Collaborative Research Group (1993) *Cell* **72**, 971–983
2. Harper, P. S. (1991) Huntington's Disease, W. B. Saunders, London
3. Martin, J. B., and Gusella, J. (1986) *N. Engl. J. Med.* **315**, 1267–1276
4. Reiner, A., Albin, R. L., Anderson, K. D., D'Amato, C., Penney, J. B., and Young, A. B. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85**, 5733–5737
5. Andrew, S. E., Goldberg, Y. P., Kremer, B., Håkan, T., Theilmann, J., Adam, S., Starr, E., Squitieri, F., Lin, B., Kalchman, M. A., Graham, R. K., and Hayden, M. P. (1993) *Nat. Genet.* **4**, 398–403
6. Snell, R. G., MacMillan, J. C., Cheadle, J. P., Fenton, I., Lazarou, L. P., Davies, P., MacDonald, M. E., Gusella, J. F., Harper, P. S., and Shaw, D. J. (1993) *Nat. Genet.* **4**, 393–397
7. Coyle, J. T., and Puttfarcken, P. (1993) *Science* **262**, 689–695
8. Beal, M. F., Kowall, N. W., Ellison, D. W., Mazurek, M. F., Swartz, K. J., and Martin, J. B. (1986) *Nature* **321**, 168–171
9. Ham, J., Babij, C., Whitfield, J. Pfarr, C. M., Lallemand, D., Yaniv, M., and Rubin, L. L. (1995) *Neuron* **14**, 927–939
10. Xia, Z., Dickens, M., Raingeaud, J., Davis, R. J., and Greenberg, M. E. (1995) *Science* **270**, 1326–1330
11. Schwarzschild, M. A., Cole, R. L., and Hyman, S. E. (1997) *J. Neurosci.* **17**, 3455–3466
12. Yang, D. D., Kuan, C.-Y., Whitmarsh, A. J., Rincon, M., Zheng, T. S., Davis, R. J., Rakis, P., and Flavell, R. (1997) *Nature* **389**, 865–869
13. Hammond, D. N., Lee, H. J., Tonsgard, J. H., and Wainer, B. H. (1990) *Brain Res.* **512**, 190–200
14. Liu, Y. F., Deth, R. C., and Devys, D. (1997) *J. Biol. Chem.* **272**, 8121–8124
15. Trottier, Y., Devys, D., Imbert, G., Saudou, F., An, I., Lutz, Y., Weber, C., Agid, Y., Hirsh, E. C., and Mandel, J. L. (1995) *Nat. Genet.* **10**, 104–110
16. Sarin, A. L. Wu, M.-L., and Henkart, P. A. (1996) *J. Exp. Med.* **184**, 2445–2449
17. Rodriguez, I., Matsuura, K., Ody, C., Nagata, S., and Vassalli, P. (1996) *J. Exp. Med.* **184**, 2067–2072
18. Gavrieli, Y., Sherman, Y., and Ben-Sasson, S. A. (1992) *J. Cell Biol.* **119**, 493–501
19. Tapon, N., Nagata, K., Lamarche, N., and Hall, A. (1998) *EMBO J.* **17**, 1395–1404
20. Vaux, D. L., and Strasser, A (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 2239–2244
21. Sanchez, I., Hughes, R. T., Mayer, B. J., Yee, K., Woodgett, J. R., Avruch, J., Kyriakis, J. M., and Zon, L. I. (1994) *Nature* **380**, 75–79
22. Lin, A., Minden, A., Martinetto, H., Claret, F.-X., Lange-Carter, C., Mercurio, F., Johnson, G. L., and Karin, M. (1995) *Science* **268**, 286–290
23. Yan, M., Dai, T., Deak, J. C., Kyriakis, J. M., Zon, L. I., Woodgett, J. R., and Templeton, D. J. (1994) *Nature* **372**, 798–800
24. Portera-Cailliau, C., Hedreen, J. C., Price, D. L., and Koliatsos, V. E. (1995) *J. Neurosci.* **15**, 3775–3787
25. Thomas, L. B., Gates, D. J., Richfield, E. K., O'Brien, T. F., Schweitzer, J. B., and Steindler, D. A. (1995) *Exp. Neurol.* **133**, 265–272
26. Bossy-Wetzel, E., Bakiri, L., and Yaniv, M. (1997) *EMBO J.* **16**, 1695–1709
27. Beal, M. F. (1995) *Ann. Neurol.* **38**, 357–366
28. Anderson, A. J., Cummings, B. J., and Cotman, C. W., (1994) *Exp. Neurol.* **125**, 286–295
29. Hunot, A., Brugg, B., Ricard, D., Michel, P. P., Muriel, M.-P., Ruberg, M., Faucheux, B. A., Agid, Y., and Hirsch, E. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94**, 7531–7536
30. Herdegen, T., Claret, F.-X., Kallunki, T., Martin,-Villalba, A., Winter, C., Hunter, T., and Karin, M. (1998) *J. Neurosci.* **18**, 5124–5135